Supplementary Information

Derlin-1 is a rhomboid pseudoprotease required for the dislocation of mutant α-1-antitrypsin from the endoplasmic reticulum

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Supplementary information comprises:

Supplementary Figures 1–6

Supplementary References

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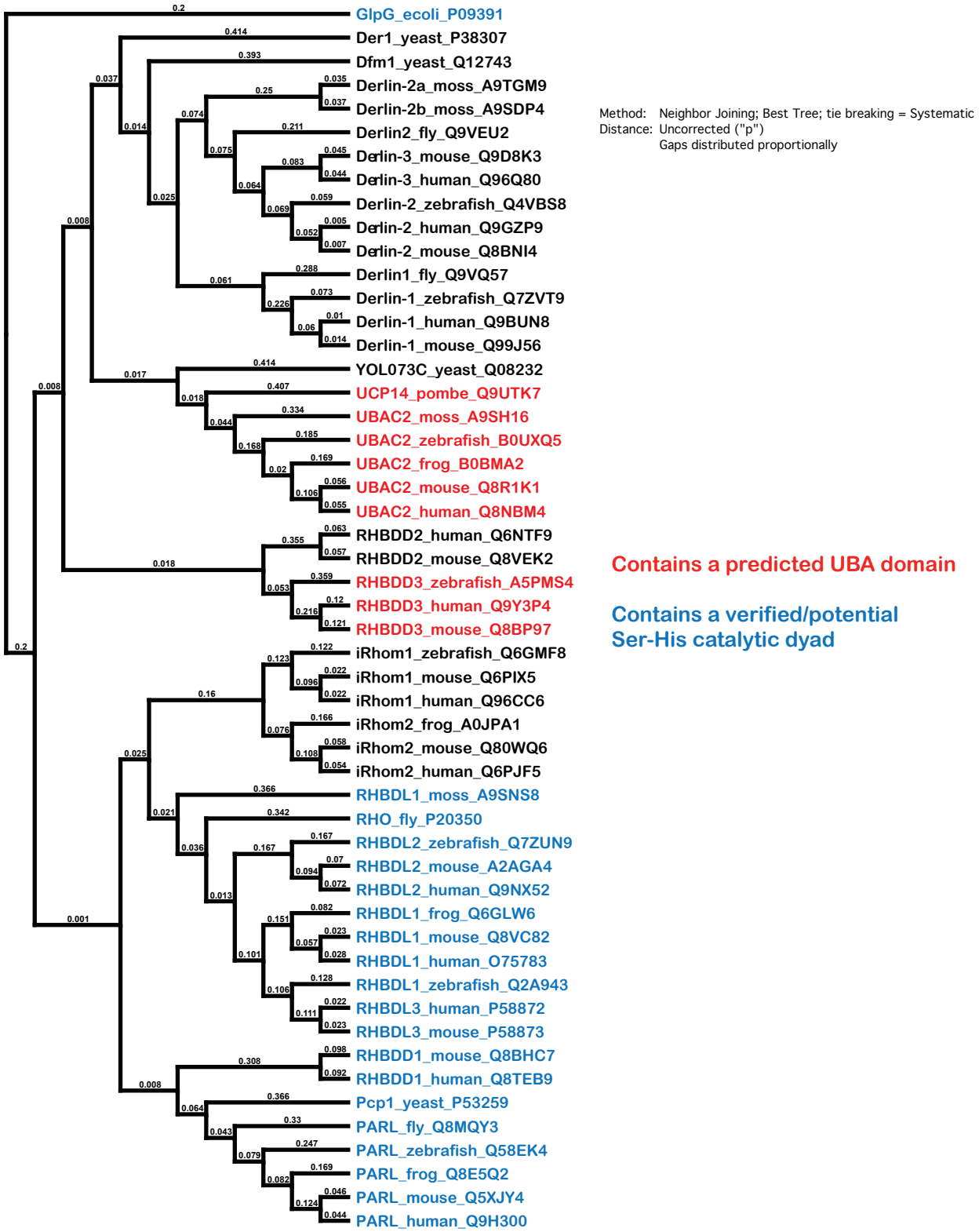
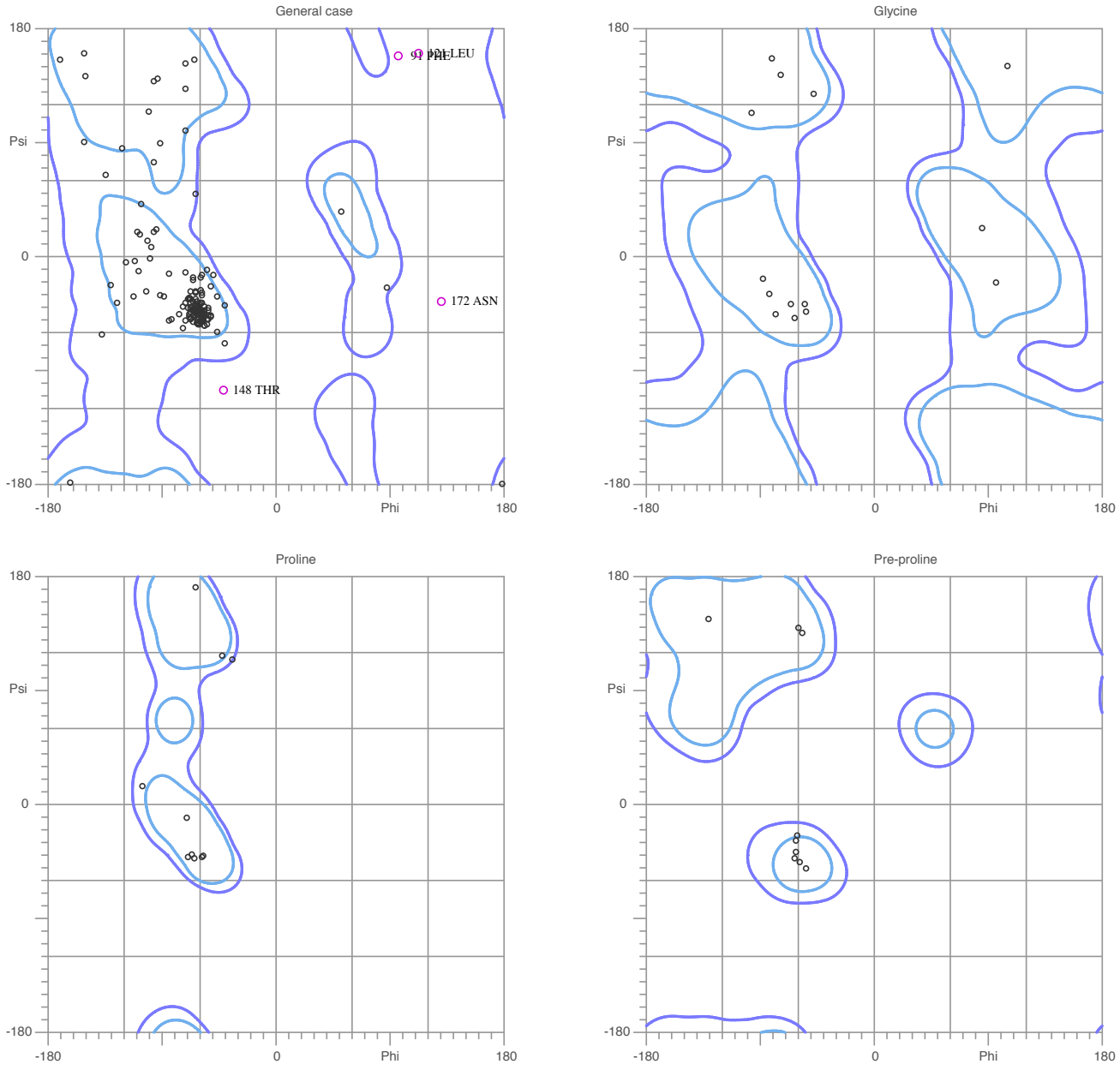
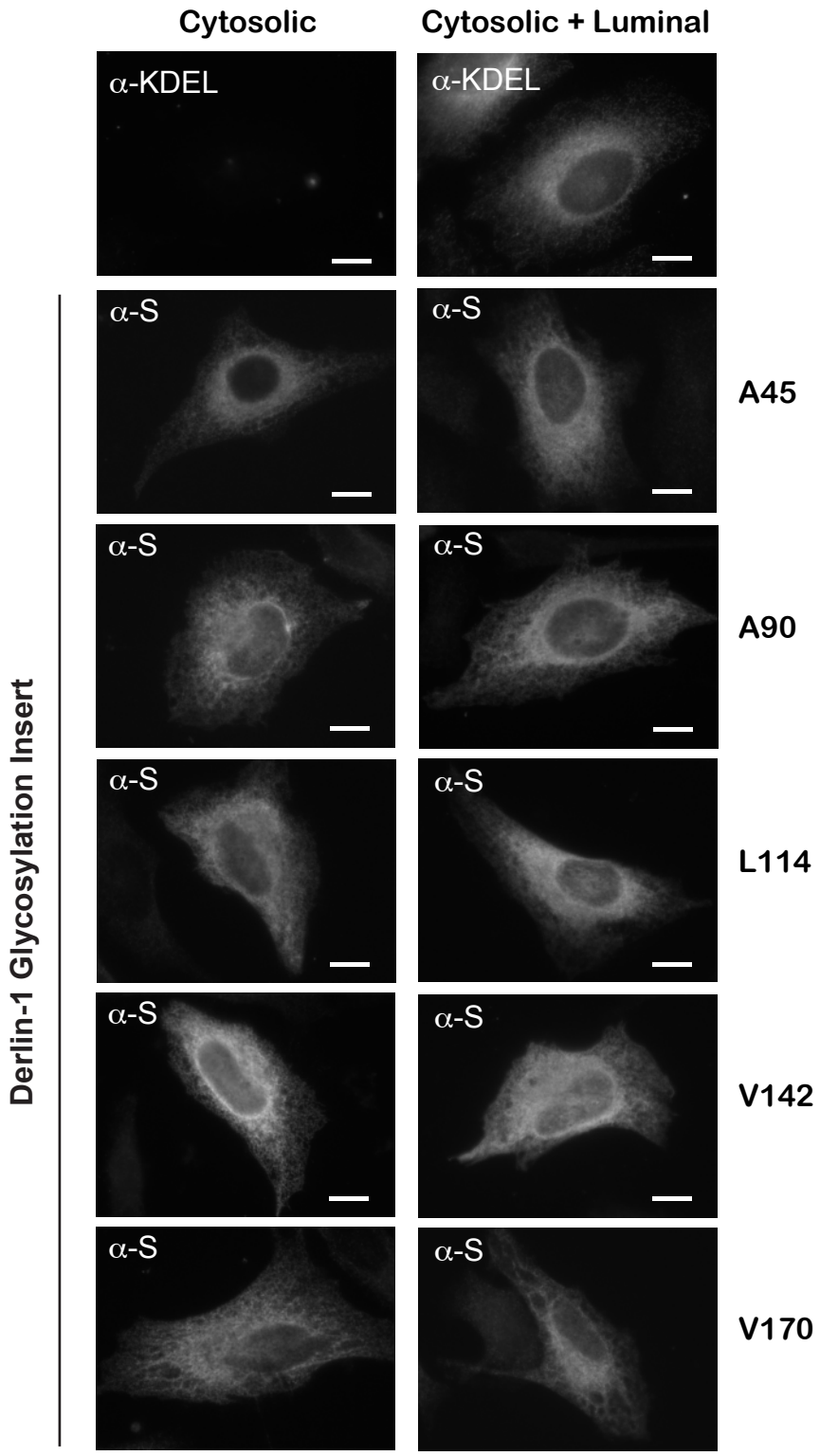


Supplementary Figure 1. Phylogenetic analysis of rhomboid family proteins. Sequences of eukaryotic rhomboid family proteins with the indicated UniProtKB accession numbers were obtained from the NCBI RefSeq database[2] and aligned using the multiple sequence and structure alignment server PROMALS3D. A phylogram was constructed by neighbor-joining using MacVector software with *E. coli* GlpG serving as an outgroup.



90.4% (161/178) of all residues were in favored (98%) regions.
97.8% (174/178) of all residues were in allowed (>99.8%) regions.

Supplementary Figure 2. Validation of the Derlin-1 homology model. Shown is the output of Ramachandran plot analysis by MolProbity of the Derlin-1 homology model depicted in Fig. 1b–d. The four outliers, F91, L121, T148, and N172 are each found in disordered loop regions connecting transmembrane spans.



Supplementary Figure 3. Insertion of a 35 amino acid N-glycosylation acceptor sequence does not perturb the overall topology of Derlin-1. HeLa cells were transfected with the indicated chimeric Derlin-1 constructs used in Fig. 1g. Cells were treated with 20 μM digitonin to permeablize the plasma membrane only (Cytosolic) or with 20 μM digitonin + 0.1% Triton X-100 to additionally permealize the ER (Cytosolic + Luminal), and stained with antibodies against KDEL or against S-tag, targetting C-terminally tagged Derlin-1-S.

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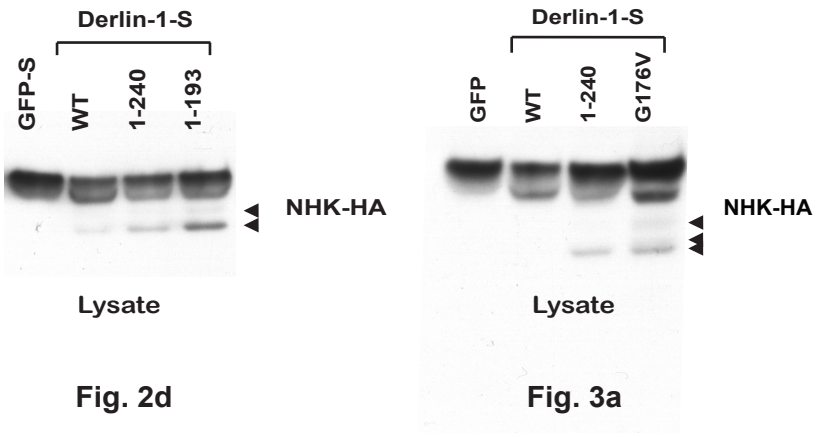
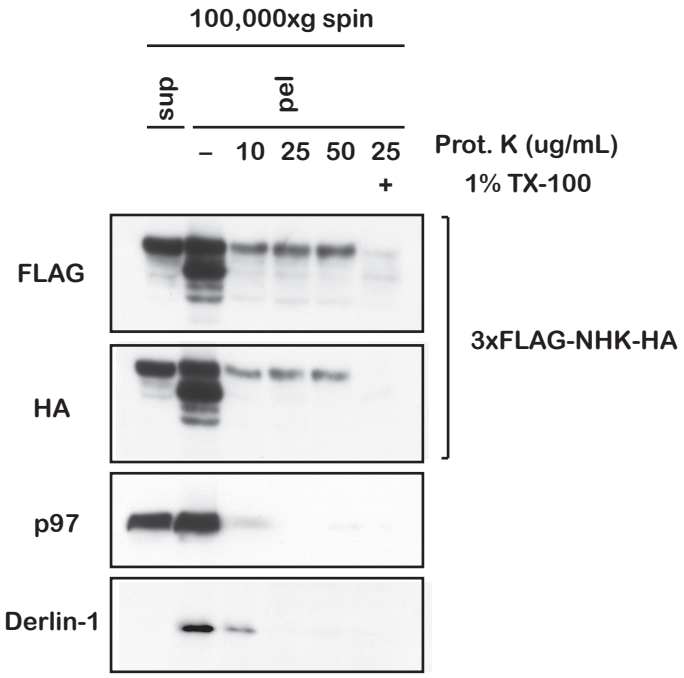


Supplementary Figure 4. Light exposures of immunoblots from Fig. 2d and Fig. 3a. The shorter-length exposures of immunoblots from the indicated figures are shown above. Arrowheads indicate deglycosylated NHK-HA.



Supplementary Figure 5. Expression of Derlin-1$_{G176V}$ stabilizes NHK on the cytoplasmic face of the ER. Membranes from cells co-expressing S-tagged Derlin-1$_{G176V}$ and N- and C- terminally tagged 3xFLAG-NHK-HA were collected and proteolyzed as in Fig. 3c. In addition, the supernatant was collected following homogenization of cells and 100,000*g* centrifugation. Proteins in this fraction, which include cytosolic as well as non-membrane-associated luminal ER proteins released during homogenization, were precipitated with 15% TCA. Equal fractions of supernatant and membrane samples were analyzed by immunobloting.

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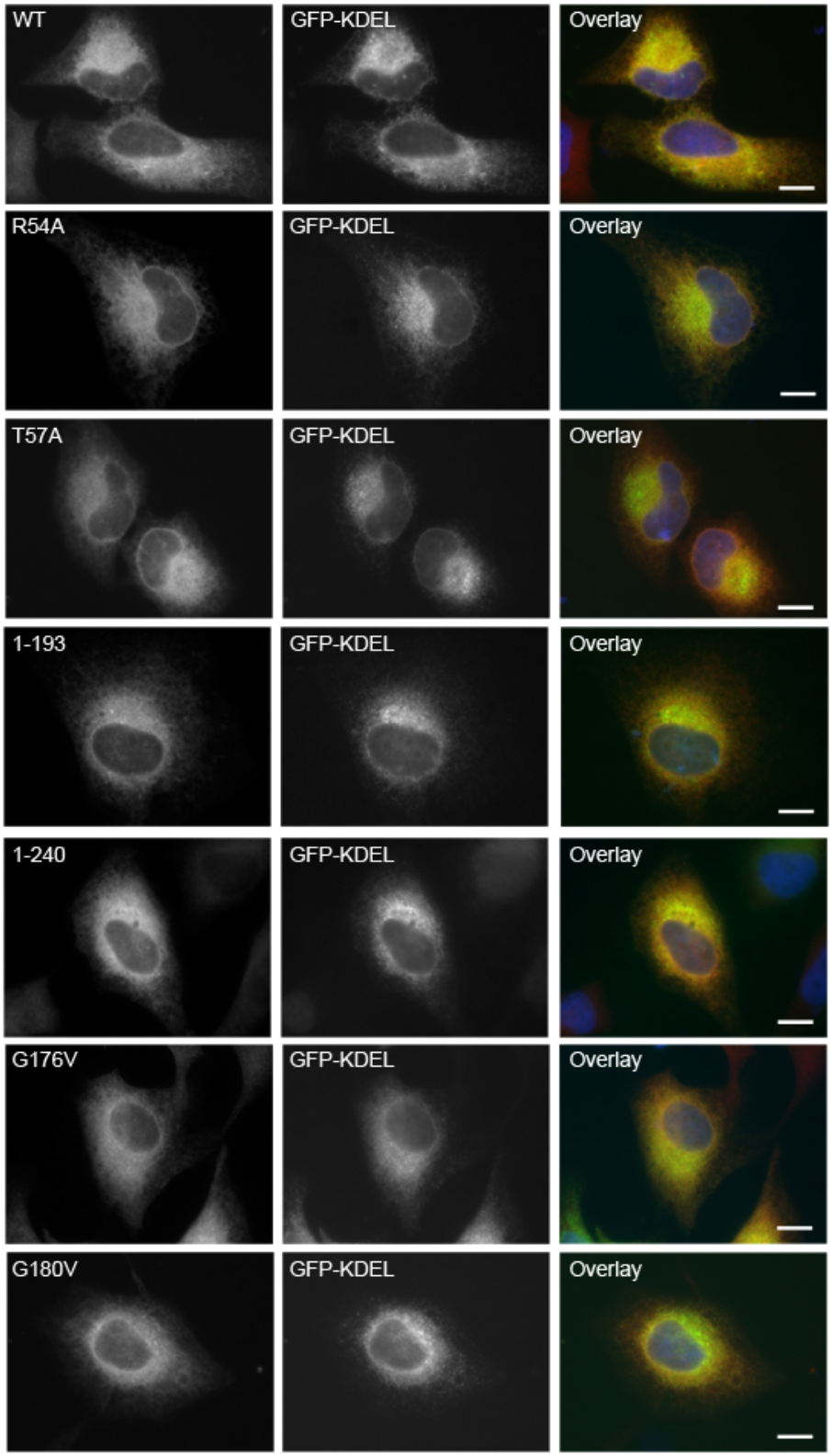

Supplementary Figure 6. S-tagged wild-type and mutant Derlin-1 localize properly to the endoplasmic reticulum. The indicated S-tagged Derlin-1 variants were co-expressed with the ER marker GFP-KDEL[1] in HeLa cells. Cells were analyzed by immunofluorescence with antibodies against S-tag.

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Supplementary References

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2 Pruitt, K. D., Tatusova, T., Klimke, W. & Maglott, D. R. NCBI Reference Sequences: current status, policy and new initiatives. *Nucleic Acids Res* **37**, D32-36, doi:10.1093/nar/gkn721 (2009).

3 Pei, J., Tang, M. & Grishin, N. V. PROMALS3D web server for accurate multiple protein sequence and structure alignments. *Nucleic Acids Res* **36**, W30-34, doi:10.1093/nar/gkn322 (2008).